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ON THE RELATION BETWEEN OXYGEN IN WATER AND THE LONGEVITY OF THE TYPHOID BACILLUS.

GEORGE C. WHIPPLE AND ANDREW MAYER, JR.

THERE are many factors which affect the longevity of the typhoid bacillus in water. Experiments which we have made at various times during the last two years indicate that the presence of dissolved oxygen is one of the most important. Comparative experiments made by putting typhoid bacilli into two portions of water and maintaining one under anaerobic conditions, while the other remained oxygenated, indicated that the bacilli preserved their vitality for a much longer period when oxygen was present. This was found to be true also of the colon bacillus.

The experiments were varied in a number of ways, as shown by the following tables. The culture of typhoid bacillus, used in all the experiments, was obtained from Dr. Ezra Wilson of the Hoagland Laboratory. Before using it, it was submitted to preliminary cultivation in broth for 24 hours at 37° C. From the third generation in broth an agar streak culture was made, which was used in subsequent inoculations.

EXPERIMENT I.*

Brooklyn tap water was filtered through a Berkefeld filter, and 19 c.c. were put into test tubes and sterilized. To each of these tubes was then added 1 c.c. of a suspension of typhoid bacilli in water. Another series of tubes was prepared in a similar way, but the water was boiled to expel the dissolved oxygen. The tubes of this series were inoculated with typhoid bacilli as in the previous case.

The first set of tubes was placed in the 20° incubator; the second was also placed in the incubator, but was kept in a Novy jar in an atmosphere of hydrogen. After various periods of incubation, one tube of each set was withdrawn and samples were plated. The following table represents the results obtained:

* Made with the assistance of Mr. Luther R. Sawin, Bacteriologist, Dept. of Water Supply, Gas, and Electricity.

DATE	PERIOD IN DAYS	TUBES KEPT IN AIR		TUBES KEPT IN HYDROGEN	
		Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
1903					
April 13.....	0	600,000	100.0	600,000	100.0
April 15.....	2	455,000	76.0	2,400	0.4
April 17.....	4	190,000	32.0	25	0.004
April 21.....	8	120,000	20.0	0	0.0
April 25.....	12	67,000	11.0	0	0.0
May 1.....	18	25,000	4.2	0	0.0
May 9.....	26	9,250	1.5	0	0.0
May 16.....	33	2,150	0.6	0	0.0
May 23.....	40	132	0.02	0	0.0
May 30.....	47	6	0.001	0	0.0
June 6.....	54	0	0.000	0	0.0

EXPERIMENT 2.

The second experiment differed slightly from the first one. Sterilized distilled water was used instead of tap water, 150 c.c. was the quantity taken, and flasks were employed instead of test tubes. The water was not boiled. Carbonic acid was used in the Novy jar instead of hydrogen. The results obtained are given in the following table:

DATE	PERIOD IN DAYS	TUBES KEPT IN AIR		TUBES KEPT IN CARBONIC ACID	
		Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
1903					
July 18.....	0	400,000	100.0	400,000	100.0
July 20.....	2	265,000	66.0	110,000	27.5
July 22.....	4			1,500	0.38
July 26.....	8	50,000	12.5	0	0.0

EXPERIMENT 3.

The experiment was made as above described except that an atmosphere of nitrogen, obtained by using pyrogallic acid and caustic potash, was used for the anaerobic flasks. The results are shown in the table following:

Period of Time	Kept in Air Number per c.c.	Kept in Nitrogen Number per c.c.
0 hours.....	3,120,000	3,120,000
24 "	No	0
48 "	record	0
72 "	kept	0

EXPERIMENT 4.

This experiment was precisely like No. 3. The results obtained were as follows:

PERIOD OF TIME	TUBES KEPT IN AIR		TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
0 hours.....	880,000	100	1,390,000	100
24 "	710,000	81	0	0

EXPERIMENT 5.

This experiment was like No. 3 except that no control culture was made in air. The results were as follows:

PERIOD OF TIME	TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number
0 hours.....	164,000	100.0
2 "	36,000	23.0
4 "	12,240	7.5
24 "	0	0.0

EXPERIMENT 6.

This experiment differed from the preceding in that 1 c.c. of the broth culture was used to inoculate the water. This apparently produced a culture medium in which the bacilli could multiply. It was observed, however, that after multiplication had taken place the numbers fell off more rapidly in the anaerobic jar. The results were as follows:

Period of Time	Tubes Kept in Air Number per c.c.	Tubes Kept in Nitrogen Number per c.c.
0 hours.....	1,500,000	1,500,000
24 "		3,000,000
7 days.....	7,200,000	4,000,000
21 "	5,000,000	550,000

EXPERIMENT 7.

This experiment was conducted like the others, but *B. coli* was used instead of *B. typhosus*. The results obtained were as follows:

PERIOD OF TIME	TUBES KEPT IN AIR		TUBES KEPT IN NITROGEN	
	Number per c.c.	Per Cent of Initial Number	Number per c.c.	Per Cent of Initial Number
Jan. 1-28, 1905				
0 hours.....	3,400,000	100	3,400,000	100.0
12 "			648,000	19.0
24 "	620,000	18	288,000	8.5
72 "			10,500	0.3

If it be true, as indicated by these experiments, that the presence of dissolved oxygen in water causes typhoid fever bacilli to maintain their vitality for longer periods of time than when no oxygen is present, this throws some light on the results of various observations which have been made by other investigators. For instance, Jordan, Russell, and Zeit¹ found by their experiments on the waters of the Chicago Drainage Canal and the Illinois River that typhoid bacilli disappeared more quickly in polluted water than in relatively pure

¹ *Jour. Infect. Dis.*, 1904, 1, p. 641.

water. Among other reasons for explaining this fact, it is quite possible that the absence of oxygen in the polluted waters may have been an important influence. There seems to be some evidence that typhoid fever germs maintain their vitality more readily in the winter season than during the summer. It is worth noting in this connection that cold water contains much larger amounts of dissolved oxygen than does warm water. The effect of the septic tank on the longevity of pathogenic bacteria also involves the relation of oxygen